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Long term prostaglandin based-protocols improve the reproductive performance after timed artificial insemination in sheep

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ABSTRACT

The aim of the experiment was to evaluate the reproductive performance of ewes synchronized with two doses of prostaglandin $F_{2\alpha}$ (PG) at different intervals and inseminated at a fixed time. During the breeding season (April to June), 370 multiparous Corriedale ewes were assigned to five groups according to body condition score and body weight, and synchronized with two doses of PG administered 7, 10, 12, 14 or 16 days apart (groups PG7, PG10, PG12, PG14 or PG16; n = 73, 76, 74, 72, 75; respectively). Cervical timed artificial insemination (Day 0) was performed at 48 ± 1.0 h (group PG7) or 56 ± 1.0 h (groups PG10, PG12, PG14 and PG16) after the second PG injection, with diluted fresh semen pooled from six adult rams. The percentage of ovulating ewes after the second PG injection and the ovulation rate (number of corpus luteum/ovulating ewes) were assessed on Day 10 by trans-rectal ultrasonography. The rate of non return to service (ewes not returning to service/inseminated ewes × 100; NRR-21) was evaluated using painted vasectomized rams. Pregnancy rate (pregnant ewes/inseminated ewes × 100) and prolificacy (foetuses/pregnant ewes) were determined on Day 60 by trans-abdominal ultrasonography. Higher NRR-21 and pregnancy rates was observed in groups PG12 (46.0%, 46.0%), PG14 (59.7%, 56.9%) and PG16 (58.7%, 56.0%) compared to PG7 (30.1%, 28.8%) and PG10 (30.3%, 30.3%; respectively $P < 0.05$). No significant differences were observed in the percentage of ovulating ewes, ovulation rate and prolificacy among groups ($P > 0.05$). Under the condition of this trial, 12, 14 or 16 days interval between PG injections enhances the pregnancy rate of ewes at cervical timed artificial insemination with fresh semen.

1. Introduction

The use of prostaglandin $F_{2\alpha}$ and its synthetic analogues (PG) in sheep reproduction programs have been studied extensively since its discovery in 1970 as a powerful luteolytic agent [1] and, to our knowledge, PG has been used to synchronize ovulation associated to timed artificial insemination (TAI) since 1978 [2]. Due to its rapid rate of lung metabolization [3,4], PG represents a better alternative for the reproductive management of the flock than sponges impregnated with progestagens [5–7].

In spite of the high oestrus response achieved following a PG synchronization protocol, variable fertility rates have been reported under different experimental conditions in ewes artificially inseminated at a fixed time [8–15]. Alterations in the contractions of the myometrium [16], sperm transport [17–20], and variability in the timing of onset of oestrus and ovulation [9,21–23] have been reported as the sources of the reproductive failure when using of PG. After finding that a 3 days old ovine corpus luteum is sensitive to PG [24], a new protocol based on two PG injections administered 7 days apart was developed (Synchrovine® protocol; [25,26]). The second PG injection applied in the early luteal phase (during the growing phase of the dominant follicle of the first wave of the cycle), induced a consistently synchronized interval from the end of the treatment to ovulation [7,24], but the pregnancy rates were low [11–13]. These poor results were associated with an altered progesterone profile that determined lower ovulation rate, fertility and prolificacy compared to a spontaneous oestrus [12]. Alternatives to improve this protocol based on promoting a surge of LH to induce and synchronize ovulation (the use of male effect or GnRH injection), different AI pathways (cervical or intrauterine), decreasing

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the PG dose or increasing intervals between PG injections (7–8 days apart) [7,14,27–29] were used, but they have been unsuccessful.

It has been suggested that the interval between PG injections should not be less than 11 to 14 days [2,30,31]. Furthermore, a decrease in fertilization rate was reported when the interval between PG injections was reduced from 14 to 8 days [32]. Administration of two PG injections 10, 12, 14 or 16 days apart lead to different durations of the luteal phase and progesterone concentrations prior to service, which were positively associated with oestradiol production by the pre-ovulatory follicles and with the pregnancy rate obtained following AI with fresh semen after oestrus detection [33]. On the other hand, the shorter PG intervals (10 and 12 days) were associated with improved oestrus response and synchrony [33], which is very important for TAI programs. Therefore, it is necessary to determine the reproductive performance achieved when these protocols are used in a TAI program.

The aim of this study was to determine the percentage of ovulating ewes, ovulation rate, rate of non return to service, pregnancy rate and prolificacy after TAI of ewes synchronized with two PG injections applied at different moments during the luteal phase under the same experimental conditions (breed, photoperiod, nutrition and health management). We hypothesized that despite the lesser oestrus response and synchrony, longer PG intervals would be associated to a greater reproductive performance after TAI. To our knowledge, this is the first study that compares the reproductive performance of different PG based protocols for TAI applying the PG injections at different moments during the luteal phase in randomly cycling ewes.

2. Materials and methods

The experiment was carried out at Escuela Agraria “La Carolina”, Flores, Uruguay (33° S-57° W), during the breeding season (April to June). The experimental procedures were approved by the Universidad de la República's Animal Ethics Committee (CUEA-Universidad de la República, Facultad de Veterinaria, Exp: 111400-000079-12).

2.1. Animals and nutrition management

Multiparous (older than 2.5 year old, and at least one parturition; n = 370) Corriedale ewes with a moderate body condition score (mean ± SD, 3.2 ± 0.4; scale 0–5, [34]) and weighing 54.5 ± 6.3 kg were used. Ewes were maintained under field conditions grazing native pasture with more than 600 kg of DM available per hectare (8% CP and 8.5 MJ ME/kg DM) and water available *ad libitum.*

2.2. Experimental design

Ewes were stratified by body condition score and body weight, then assigned randomly to five groups synchronized with two injections of Delprostenate im (160 μg per injection, Glandinex®, Universal Lab, Montevideo, Uruguay), administered 7, 10, 12, 14 or 16 days apart (PG7, PG10, PG12, PG14 or PG16 respectively; n = 73, 76, 74, 72 and 75 ewes respectively). The average body condition score of each group was 3.2 ± 0.4, 3.2 ± 0.4, 3.2 ± 0.4, 3.2 ± 0.4 and 3.2 ± 0.3 (PG7, PG10, PG12, PG14 and PG16 respectively). Each group received the first injection of PG on a different day in order to perform the second PG injection and TAI of all groups on the same day (a schematic representation of the experimental design is shown in Fig. 1). In order to reach the adequate insemination time, using the same semen and made the insemination randomly between groups, the ewes of PG7 group received the second PG injection 8 h later to the remains groups.

2.3. Semen collection, evaluation and dilution

Semen from six adult Corriedale rams, checked for normal breeding soundness, was collected using an artificial vagina and assessed as described by Evans and Maxwell [35]. Two consecutive ejaculates from each ram were collected, evaluated and pooled according to the individual sperm concentration, so that each ram contributed with similar number of spermatozoa to the pool. Soon after pooling, the semen was extended to the final sperm concentration with UHT skim milk supplemented with 5% of egg yolk (v/v) and antibiotics (100.000 IU sodium penicillin and 100 mg of dihydrostreptomycin/100 mL of extender). Diluted semen with a minimum of 80% of progressive motility was used for TAI.

2.4. Artificial insemination

Cervical AI was performed using a speculum equipped with a light source and an insemination device (Walmur® Veterinary Instruments, Montevideo, Uruguay) as described by Evans and Maxwell [36], by two teams of technicians. Ewes were inseminated randomly among PG groups by one of the two teams of technicians that were not aware on the treatments. The insemination dose per ewe was 0.15 mL containing 160 × 10^6 spermatozoa, slowly released as deep as possible into the cervix. Extended semen was maintained at room temperature and protected from sunlight until AI. On the basis of previous reports describing ovulation and insemination time of PG protocols, ewes were inseminated at 48 ± 1.0 h after the second PG injection in group PG7 [11,13] and at 56 ± 1.0 h in groups PG10, PG12, PG14 and PG16 [10,33].

2.5. Ultrasound evaluation and non return rates

The percentage of ovulating ewes (ewes that ovulated after second PG injection/total ewes × 100), and ovulation rate (number of corpus luteum/ovulating ewes) were evaluated on Day 10 by trans-rectal ultrasonography with a 7.5 MHz linear array transducer (ALOKA SSD-500, Overseas Monitor Corp. Ltd., Tokyo, Japan) as described by Viñoles et al. [37]. In order to calculate the rate of non return to service up to Day 21 (ewes not returning to service/inseminated ewes × 100; NRR-21) oestrus was detected daily (from 18:00–08:00 h the following morning) from Day 13 to 21, with painted Corriedale vasectomized rams at a rate of 3 rams/100 ewes. In order to calculate pregnancy rate (pregnant ewes/inseminated ewes × 100) and prolificacy (foetuses/pregnant ewes) pregnancy was diagnosed and foetuses counted on Day 60 by ultrasonography with a 3.5 MHz convex array trans-abdominal transducer (ALOKA, Tokyo, Japan).

2.6. Statistical analyses

Data were analyzed by logistic regression using the Genmode procedure in SAS (SAS 9.2V; SAS Institute Inc., Cary, NC, USA), considering a binary response (0 and 1 or ≥1) and treatment as the explanatory variable. Ovulation rate and prolificacy are presented as means ± SD, ovulating ewes, NRR-21 and pregnancy are presented as percentage. Differences were considered significant if $P < 0.05$.

3. Results

The rates of non return to service up to Day 21 and pregnancy rate of groups PG12, PG14 and PG16 were higher than groups PG7 and PG10 ($P < 0.05$; Table 1). No differences were found in these